

CHAPTER 2

ELECTRON TRANSFER REACTIONS OF VITAMIN E

2.1 INTRODUCTION

2.1.1 Free Radical and Electron Transfer Reactions in Organic Chemistry

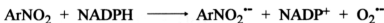
Organic reactions can occur *via* many types of mechanisms involving various species such as neutral molecule, ion or free radical. A free radical can be an atom, molecule, molecular fragment or complex compound which possesses at least one unpaired electron. Radicals are relatively unstable and reactive entities. Reactions involving radicals are very fast processes and can provide a wide range of applications in organic synthesis. Generally, a radical chain reaction proceeds in three phases, i.e. initiation, propagation and termination. In the initiation, free radicals can be generated from molecules by radiation (such as heat, ultraviolet, microwave, X-rays, electrons, α -particles, neutrons and γ -rays), redox processes, electron transfer or mechanical degradation, all of which can cause cleavage of bonds; electrochemical processes also produce cationic or anionic radicals. A few types of propagation steps involving radical and non-radical species have now been identified in chain reaction systems including addition, substitution (or abstraction), elimination (or fragmentation), rearrangement and electron transfer [209]; these processes while consuming radicals are also generating new radicals and therefore will maintain the chain propagation. In chain-terminating steps, combination or coupling of two radicals will lead to bond formation and give rise to non-radical products or disproportionation may also occur to yield two non-radical products. Electron transfer is one class of oxidation-reduction reaction [150] which is of importance in chemistry and other fields of science. Single electron transfer (SET) is a fundamental process that occurs in many organic reactions; this is an outer sphere (non-bonding) electron transfer process in which radicals and radical ions are usually formed [454]. Qualitative and quantitative understanding of electron transfer for many reaction mechanisms have been studied by optical rotation, nuclear magnetic resonance (NMR), electron spin resonance (ESR) and isotopic-tracer methods. It is now known that nucleophiles and strong bases can react with weak organic oxidants by single electron

transfer process instead of a conventional two-electron addition, substitution or deprotonation.

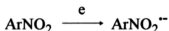
2.1.2 Consequences of Free Radical Attack in Biological Systems

In biological systems, the major reactions involving active oxygen and free radicals can be found in the following examples: (i) initiation of autoxidation chain processes by hydroxyl and hydroperoxyl radicals, and branching reactions by alkoxyl radicals; (ii) addition of hydroxyl radicals and singlet oxygen to double bonds; (iii) hydrogen abstraction from allylic carbon atoms by hydroxyl radicals; (iv) oxidation of sulfhydryl, thioester and amino functions, etc. [522]. The biological consequences of these radical processes include mutation, chromosomal aberrations, cytotoxicity, carcinogenesis, and cellular degeneration related to ageing, etc.

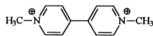
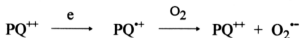
It is now known that a large number of drugs and toxins can interrupt cellular electron flow and generate free radicals which may affect the drug's action or toxicological consequences. For example, nifurtimos (a nitrofurane derivative, ArNO_2) [142] and bleomycin (BLM, an anti-tumour antibiotic drug used in cancer therapy) [281] appear to function by electron transfer and generate superoxide and hydrogen peroxide.



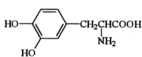
Certain nitroaromatic compounds (ArNO_2) used as radiation sensitizers or drugs to increase the sensitivity of tumours to radiation therapy [49,50] also act through single electron transfer mechanism.



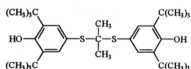
Paraquat (PQ) is a bipyridylum salt used as herbicide which acts as an electron acceptor, it can readily undergo an electron transfer cycle and continuously generates superoxide radical anion, therefore may cause uncontrolled free radical lipid peroxidation and toxicity [91].



Paraquat

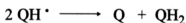
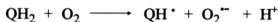


Dopa



probucol

The oxidation of hydroquinone-type compounds (QH_2) also produce superoxide *via* single electron transfer steps which are important features for a number of drugs including Dopa [23-25,68]. Probucol is another powerful inhibitor of lipid peroxidation in rat liver microsomes, it can also undergo one electron-oxidation [10].



Role of active oxygen and radicals in carcinogenesis

The observation of the tumour promoting activity of dioxygen (O_2), superoxide ($\text{O}_2^{\cdot -}$) and some organic hydroperoxides support the view that active oxygen plays an important role in carcinogenesis. These reactive species or their parent compounds could promote chemically or radiation-initiated transformation of normal cells into cancerous cells [556,582,707]. The carcinogenic properties exhibited by a wide range of polynuclear aromatic hydrocarbons (PAH, e.g. benzo[a]pyrene) have been attributed to their involvement in radical formation [521] through single electron transfer system which give

a variety of products including phenols and quinones [535,644]. Another mechanism is that the enzymatically catalyzed conversion of PAH to carcinogens involves epoxidation of the aromatic ring by peroxy radicals or P-450 system and produce primarily arene oxides and diols, e.g. 7,8-diol of benzo[a]pyrene to diol-9,10-epoxide, and further metabolized to 7,8,9,10-tetraol by a prostaglandin-oxidizing and hydrolase systems [398].

2.1.3 Mechanisms for the Reactions of Some 4-Nitro-Compounds with Base

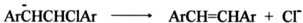
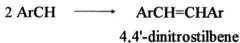
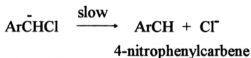
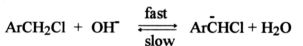
Mechanistic studies provide the understanding of how the individual steps of a reaction proceed. One of the systematic and well studied mechanisms in organic chemistry is the reaction of arylalkyl halides with anions or bases. The reaction of 4-nitrobenzyl derivatives with base (first observed in the 1890's) has been one extensively studied reaction. A variety of mechanisms have been postulated and developed based on various reaction conditions and these include S_N2 [489,490], carbanion [243,345,572], carbene [2,44,244,544,545] and electron transfer [33,100,106,107] pathways.

S_N mechanism

The nucleophilic displacement process is the earliest pathway that received substantial support for the reaction of 4-nitrobenzyl halides with bases or anions. The hydrolysis of benzyl chlorides in neutral medium usually proceeds by S_N1 mechanism [489,490]. With halide leaving groups such as iodide and bromide, S_N2 displacement by hydroxide ion or methanol occurs in an ordinary manner to produce 4-nitrobenzyl alcohol and 4-nitrobenzyl ether [2]. However, in the reaction of 4-nitrobenzyl chloride with sodium hydroxide in aqueous alcohol, acetone or dioxan [244,658], 4,4'-dinitrostilbene ($ArCH=CHAr$) has been found to be the major product instead of the expected 4-nitrobenzyl alcohol as produced by alkali hydrolysis of benzyl chloride in water [54,363,387,538,593]; this indicates that a different process has occurred when bromide or iodide is replaced by a poorer leaving group.

olefins was unsuccessful in the reactions of benzyl halides with *n*-butyllithium [273] or 4-nitrobenzylidene dichloride with sodium hydroxide using phase-transfer technique [100]. Furthermore, using 4-nitrobenzylidene dichloride in attempts to produce a more stable carbene where chlorine is expected to stabilize carbene [343], products postulated from the carbene mechanism such as dimer $(\text{ArCCl})_2$ was not found [100,216], and a kinetic study indicated a second order in the starting reactant halide also ruled out the carbene mechanism [216].

Scheme 2.2 Carbene Mechanism

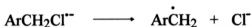


Electron transfer mechanism: radical anion as an intermediate

Until 1960's, certain substitution reactions at a saturated carbon atom were found to proceed *via* multi-stage sequences involving radical anions and free radicals as intermediates. "Radical anion" is a relatively short-lived intermediate derived from a neutral molecule upon receiving an electron from other electron donor such as carbanion. The dominant features of radical anions in various nitroaromatic systems [353,356-358,360,551] or 4-nitrobenzyl halide systems with bases have been extensively studied [177,245,354,553]. The development of free radical chain process in aliphatic systems involving the formation of radical anions has been reviewed by Kornblum [360] and

Russell [554]. Evidence accumulated especially from a series of reactions of nitroparaffin salts (e.g. salt of 2-nitropropane) with a 4-nitrobenzyl system suggested that the carbon alkylation [239] occurs *via* a radical anion process [330,331,351,551,553], and it was proven to be a chain reaction in another analogous system [354]. The radical-anion mechanism proceeds by electron transfer from 2-nitropropyl anion to 4-nitrobenzyl chloride to form 2-nitropropyl radical and 4-nitrobenzyl chloride radical anion ($\text{ArCH}_2\text{Cl}^{\cdot-}$). The resulting radical anion fragments by undergoing scission of the C-Cl bond to generate 4-nitrobenzyl radical which selectively couples with the 2-nitropropyl radical to yield carbon alkylated products [330,331,550]. The mechanism is exemplified in Scheme 2.3. Para-dinitrobenzene (*p*-DNB), a powerful electron acceptor, has been shown to suppress the carbon alkylation [330] by destroying the benzyl radical anion before loss of the chloride ion can occur [331]. The support for the radical-anion mechanism also came from the product analysis using compounds with considerable steric hindrance such as *p*-nitrocumyl halides [355,360]. The radical anion of coupling product 4-nitrobenzyl-2-nitropropyl in ethanol or *N,N*-dimethylformamide formed during photochemical coupling has also been detected by ESR [550].

Scheme 2.3 Single Electron Transfer Mechanism

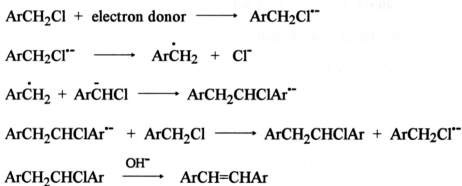


$S_{\text{RN}}1$ mechanism (chain process)

In order to explain the formation of 4,4'-dinitrostilbene from 4-nitrobenzyl chloride, combination of radical and nucleophile to produce radical anion has been proposed as exemplified in Scheme 2.4. The reaction is initiated by formation of 4-

nitrobenzyl chloride radical anion which rapidly fragments to give the 4-nitrobenzyl radical, and propagates by addition of the benzyl radical to 4-nitrobenzyl carbanion to yield a dimeric radical anion. Subsequently, the dimeric radical anion transfers its unpaired electron to another 4-nitrobenzyl chloride molecule to produce a non-radical dimeric product and at the same time regenerate the 4-nitrobenzyl chloride radical anion [331,360,361]; these steps constitute a cycle, and can also be applied to other nitroaromatic systems [8,30,145,149,374,552]. This process was originally recognized in the reactions of 2-nitropropyl anion ($\text{Me}_2\text{C}=\text{NO}_2^-$) with 4-nitrobenzyl halides and resembles the electron transfer radical mechanism for nucleophilic substitution at saturated carbon as proposed by Kornblum *et al.* [331,353,354,356,511] and Russell & Danen [550,552], and it was named as "unimolecular nucleophilic radical substitution" or $\text{S}_{\text{RN}}1$ sequence by Kim and Bunnett [342]. The conversion of 4-nitrobenzyl derivatives to stilbenes by the action of base has been proposed to proceed via $\text{S}_{\text{RN}}1$ sequence [358] with electron transfer as the key step [331,352]. The combination of a free radical with an anion is the fundamental step in $\text{S}_{\text{RN}}1$ process and is considered as the rate-determining step. The radical anion non-chain mechanism for aromatic substitution has also been modified to be a $\text{S}_{\text{RN}}1$ chain process [78,543].

Scheme 2.4 $\text{S}_{\text{RN}}1$ Mechanism

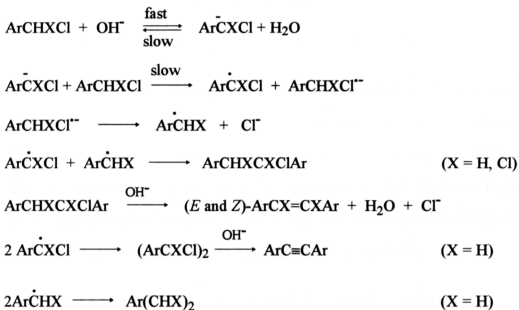


Free radical-radical anion non-chain mechanism

Although $S_{RN}1$ mechanism provides a good explanation for the production of 4,4'-dinitrostilbene in the reaction of 4-nitrobenzyl chloride with hydroxide ion, 4,4'-dinitrostilbene is not the exclusive product as previously reported; other minor products such as bis-(4-nitrophenyl)-acetylene, 4,4'-dinitrobibenzyl and 4-nitrotoluene have also been identified [106]. A modified reaction mechanism is shown in Scheme 2.5. Initially this mechanism was not favoured [2] but it is now widely accepted as a combination steps of carbanion formation, electron-transfer, halide-elimination and coupling of the resulting radicals [2,100,106,107,216,316]. Under a strong basic condition, carbanion formation is the first step and occurs much faster than the other subsequent steps [100]. The electron transfer, elimination and coupling steps in this mechanism basically resemble those first suggested by Beringer *et al.* in an analogous system (i.e. phenylation of sodium salt of 2-phenyl-1,3-indandione by diphenyliodonium chloride in *tert*-butyl alcohol) [45], and also based on some common routes established by Kornblum and coworkers for the reactions of nitrobenzyl derivatives [331,355,357]. There is compelling evidence that radical anions and free radicals are intermediates involved in the reactions of 4-nitrobenzyl derivatives with bases [106,617,423]. Second-order kinetics are consistent with the radical mechanism in which the rate-determining step is electron transfer from the carbanion to the reactant molecule [216]. Paramagnetic species present in the reactions of 4-nitrobenzyl halides with KOH have been detected by ESR [107]. Using γ -ray radiation at 77 K, it has been proven by ESR studies [617] that 4-nitrobenzyl halides are able to capture an electron to form radical anions which subsequently dissociate very rapidly to halide ions and radicals. 4-Nitrobenzyl radical (ArCH_2^\bullet) couples with α -chloro-4-nitrobenzyl radical (ArCHCl^\bullet) to give α -chloro-4,4'-dinitrobibenzyl ($\text{ArCH}_2\text{CHClAr}$) which will undergo β -elimination of a hydrogen chloride with the base giving rise to the *cis,trans*-4,4'-dinitrostilbene (ArCH=CHAr). Formation of bis-(4-nitrophenyl)-acetylene ($\text{ArC}\equiv\text{CAr}$) as a side-product is possible *via* dimerization of the α -chloro-4-nitrobenzyl radical

(ArCHCl $\dot{}$) followed by elimination of HCl. Other products such as 4,4'-dinitrobibenzyl (ArCH $_2$ CH $_2$ Ar), 4-nitrotoluene and *cis,trans*-dinitrostilbene oxides can also be explained by some intermediate steps involved in the electron transfer mechanism [245,316]. Kinetic data furnished support that electron-transfer is the rate-determining step but not the α -elimination nor S $_N$ 2 steps [107,216]. Results of the reaction using 4-nitrobenzylidene dichlorides with base confirmed the view that the radical mechanism is important in the reactions of 4-nitrobenzyl halides in the presence of base [217].

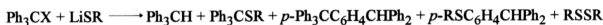
Scheme 2.5 Free Radical-Radical Anion Mechanism



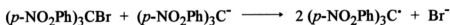
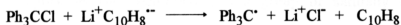
2.1.4 Electron Transfer Reaction of Triphenylmethyl Halides

Reactions of one-electron acceptors, *viz.* triphenylmethyl halides (Ph $_3$ CCl or Ph $_3$ CBr) with alkali metal amides (e.g. lithium diisopropylamide), alkoxides (e.g. LiO t Bu and KO t Bu) [13], or metal hydrides (e.g. LiAlH $_4$, MgH $_2$, etc.) [114] are among interesting systems used to study potential electron transfer processes. The detection of triphenylmethyl radical as an intermediate provides evidence that some of the reactions may involve an electron transfer mechanism [13,114]. Product analyses also indirectly

support the single electron transfer mechanism. For example, KO^tBu reacts with triphenylmethyl bromide to yield ether products mainly with α -substitution (i.e. $\text{Ph}_3\text{CO}^t\text{Bu}$) and a minor product with para-substitution, i.e. p - $^t\text{BuOC}_6\text{H}_5\text{CPh}_2$, both can be formed *via* an apparent electron transfer process [12]. Other radical products in the reaction of triphenylmethyl halide with $\text{LiN-}i\text{-Pr}_2$ are triphenylmethane (Ph_3CH) and dimerization product (4-benzhydrylphenyl)triphenyl methane (i.e. p - $\text{Ph}_3\text{CC}_6\text{H}_4\text{CHPh}_2$) derived from the triphenylmethyl radical; analogous reduction products were also obtained when lithium thiolates (LiSR) were used as one-electron donor [17].



The reaction of triphenylmethyl chloride and lithium naphthalenide ($\text{Li}^+\text{C}_{10}\text{H}_8^{2-}$) proceeds by electron transfer to produce triphenylmethyl radicals and then leads to dimers [706]. Reaction of triphenylmethyl chloride with triphenylmethyl carbanion *via* one-electron oxidation process to form triphenylmethyl radical [706] is an example analogous to the 4-nitrobenzyl halide systems. Electron transfer from tris- p -nitrophenylmethide ion to a tris- p -nitrophenylmethyl bromide has also been reported [312].



The predominant product was triphenylmethane when triphenylmethyl halides were treated with excess lithium dialkylamide or lithium *tert*-butylethylamide bases in tetrahydrofuran at -78°C [455]. The minor dimer was (4-benzhydrylphenyl)triphenyl methane which was expected to be isomerized from another trityl dimer (i.e. 1-diphenylmethylene-4-trityl-2,5-cyclohexadiene) in base-catalyzed reactions [237,595]. 1-Diphe-

nyl-methylene-4-trityl-2,5-cyclohexadiene was found to be a bimolecular coupling product from the triphenylmethyl radical [304,373]. The ESR studies [12] provide direct support that electron transfer processes occur in the reactions of lithium dialkylamide and triphenylmethyl halides in tetrahydrofuran. However, product, kinetic, kinetic isotope effect and labelling studies [455] demonstrated that the reaction proceeds *via* formation of an ion pair containing trityl-THF oxonium cation followed by diffusion controlled electron transfer from the base to the trityl-THF oxonium ion. Other reaction systems such as alkyl halides [12,18], polynuclear aromatic hydrocarbons (PAH) [16], and ketones [15] with various alkali metal hydrides or alkoxides [12] have been claimed to proceed *via* the electron transfer pathway but not by the classic polar mechanism [283,309,365]. PAH such as perylene, 2,3-benzanthracene, benzo[a]pyrene, phenanthrene, chrysene and anthracene were reported to react with lithium diisopropylamide to produce stable radical anions detectable by ESR [12].

2.1.5 Antioxidant Activity of Vitamin E in Free Radical Processes

Chemical carcinogenesis in animals is now understood to involve various chemical entities including free radicals, there being numerous reports which demonstrated that antioxidants can inhibit the growth and incidence of chemically-induced tumours. Vitamin E (*viz.* tocopherols and tocotrienols) is a group of naturally occurring chromanol compounds which can act as an efficient chain-breaking antioxidant, and it is the major lipid-soluble chain-breaking antioxidant present in biomembranes [89]. Its function as an antioxidant *via* donation of its phenolic hydrogen atom to a radical species and thus being transformed into a stable chromanoxyl radical has been detected by ESR. Among the tocopherol homologues, α -tocopherol (α -T) is the most biologically active component [83,85]; *in vitro* experiments [694] have shown that vitamin E could suppress the oxidation and hemolysis of vitamin E-deficient rat's erythrocytes and the process is accelerated by a free radical initiator. Tocotrienoxyl and tocopheroxyl radicals (TO^{\bullet}) as generated by hydrogen abstraction or electron transfer reactions using metal ions have

been recorded by ESR [219,267]. These chromanoxyl radicals have also been demonstrated to be capable of scavenging other free radicals to form non-radical products.



2.1.6 Objectives of the Present Research

The present research will probe the reaction of nitroaromatics with tocopherols and tocotrienols based on some reactions of 4-nitrobenzyl chloride with α -T and α -T₃, both derived from palm oil. Preliminary experiments used a two-phase system under strong basic condition employing benzyltriethyl ammonium chloride as the phase transfer agent. The generation of chromanoxyl radicals from the α -T and α -T₃ was examined together with the product distribution. Relative reactivities of various vitamin E components in the reaction with 4-nitrobenzyl chloride and triphenylmethyl chloride were examined for potential SET reaction.

2.2 EXPERIMENTAL

2.2.1 Chemicals and Materials

α -T and α -T₃ were isolated from a vitamin E concentrate derived from palm fatty acid distillate as described in Chapter 1. 4-Nitrobenzyl chloride of synthesis grade (Merck) was recrystallized from absolute ethanol prior to use for reaction, benzytriethyl ammonium chloride (Merck) was used as a phase transfer catalyst, sodium hydroxide (Fluka), dichloromethane (Merck), hexanes (J.T. Baker) and ethyl acetate (Merck) of analytical grade were used. Deoxygenated solvents and distilled water were prepared by passing through the previously boiled solvents with purified nitrogen gas for 30 minutes. Triphenylmethyl chloride (Fluka) was recrystallized from petroleum ether (60-80°C) before use.

2.2.2 Reactions of α -T₃ and α -T with 4-Nitrobenzyl Chloride

A 250 mL two-neck round bottom flask was charged with 2.5 mmol (1.1 g) α -T₃, 2.5 mmol (0.44 g) 4-nitrobenzyl chloride and 0.05 mmol (0.01 g) benzytriethyl ammonium chloride in 100 mL of deoxygenated dichloromethane. Under a nitrogen atmosphere, the reaction was initiated by syringing in deoxygenated NaOH (10 g in 20 mL H₂O) and efficiently stirred at room temperature for 2 hours. The reaction was monitored by TLC using UV-light and iodine staining for detection of the products. The reaction was quenched, while cooling, by addition of 10% hydrochloric acid until the reaction mixture was neutralized. Small amounts of yellow solids were filtered and washed with water. The organic layer was separated and washed with water. After the removal of solvent and dried with vacuum pump, the crude mixture was chromatographed on silica gel (Merck, 230-400 mesh) column, eluted with a hexane-ethyl acetate mixture (gradient from 98:2 to 90:10, v/v). The fractions were qualitatively examined by TLC and ¹H NMR spectroscopy. Quantitative estimations were made by weighing each fraction after removal of the solvent in conjunction with ¹H NMR spectral data.

In order to analyze the minor products, a scaled-up reaction for α -T (20 mmol) was carried out. Reaction of 5 mmol α -T (2.1 g) with 5 mmol p-dinitrobenzene (0.8 g) in dichloromethane-water was also carried out. Products were purified by silica gel column chromatography using 2 - 5% of ethyl acetate in hexanes, and characterized by ^1H and ^{13}C NMR spectroscopy.

2.2.3 Reaction of α -T with Triphenylmethyl Chloride

Triphenylmethyl chloride (4.8 mmol) and α -T (4.5 mmol) in 50 mL dichloromethane was mixed with aqueous NaOH (5 g in 25 mL), benzyl triethyl ammonium chloride (0.2 mmol) was used as phase transfer catalyst. The progress of the reaction was monitored by TLC using 30% of dichloromethane in hexanes, and examined by UV and iodine staining. When most of the α -T had reacted over two hours, the reaction was terminated by acidification using 10% HCl. In a control reaction, conditions were the same as above except that α -T was omitted.

2.2.4 Qualitative Analyses of Reaction Products

TLC was performed using Merck's silica gel 60 F₂₅₄ plate. Purified products were analyzed by ^1H and ^{13}C NMR and mass spectroscopy. ^1H and ^{13}C NMR spectra for the products dissolved in CDCl_3 were recorded on a JEOL EX90A spectrometer. For high resolution NMR analysis, JEOL JNM-GSX270 FT NMR spectrometer was used. Chemical shifts are reported as δ_{H} ppm downfield from tetramethylsilane (TMS) as internal standard in ^1H NMR data, whereas $\delta_{\text{C}} = 77.00$ ppm was used as the reference peak of chloroform in ^{13}C NMR data. Mass spectra of pure samples were recorded with Fisons ProSpec spectrometer employing the EI mode.

Chemical shifts of carbons in the phytol side chain of α -T and the isoprenoid side chain of α -T₃ fragments in the reaction products are assigned based on the previous report [76]. Carbons in the chromanyl moieties are assigned with reference to previous reports [219,267] and by 2D-NMR.

2.2.5 Kinetic Experiments

Crude kinetic experiments were carried out at room temperature (300 K) to study the reactivity of vitamin E with various electron acceptors. Equal molar ratio (0.75 mmol) of α -T or α -T₃ for reactions with 4-nitrobenzyl chloride, triphenylmethyl chloride or *p*-dinitrobenzene were dissolved in 7.5 mL of dichloromethane and mixed with 4 mL of 5 M aqueous NaOH, the mixtures were efficiently stirred under nitrogen atmosphere. Aliquots were taken at intervals (0, 10, 15, 30, 45, 60, 120, 150, 180, 240, and 300 min), acidified with 10% aqueous HCl, the organic layer was flushed with nitrogen gas and moisture was removed by a vacuum pump. The product mixtures in CDCl₃ were analyzed by a 270 MHz NMR spectrometer. The disappearance of 4-nitrobenzyl chloride and the formation of reaction products were followed by ¹H NMR spectroscopy measuring the intensities of aromatic protons and methylene protons derived from 4-nitrobenzyl chloride. The disappearance of reactant α -T in the reactions with 4-nitrobenzyl chloride and triphenylmethyl chloride were also monitored by measuring the intensities of its aromatic methyl groups which was distinguishable from those in the products.

2.3 RESULTS AND DISCUSSION

2.3.1 Reactions of α -T₃ and α -T with 4-Nitrobenzyl Chloride

The reactions of α -T₃ or α -T with 4-nitrobenzyl chloride were carried out at room temperature in the presence of NaOH. The products were purified by silica column chromatography, spectral and other data of the products are tabulated in Table 2.1, and their structures are shown in Fig. 2.1. ¹³C and ¹H NMR spectral data and their assignments are given in Tables 2.2 and 2.3, respectively. The major product is an ether cross product expected from the coupling of 4-nitrobenzyl chloride with α -T₃ or α -T, these compounds were identified as 4-nitrobenzyl- α -tocotrienol ether (**1a**) and 4-nitrobenzyl- α -tocopherol ether (**1b**) with a yield of 70% and 72% (based on α -vitamer), respectively. Smaller amounts (ca. 4%) of isomeric carbon-carbon cross products **2a** & **3a** and **2b** & **3b** have also been obtained from the respective reactions. Minor products obtained from the reaction of α -T with 4-nitrobenzyl chloride are **4** (0.4%, an isomer of **3b**) and **5** (1%) as shown in Fig. 2.1, trace amount of **6** (Fig. 2.4) has also been obtained; although these minor products were also expected from the reaction using α -T₃ but their quantities were inadequate for detailed characterization. In the presence of α -T₃ or α -T, only minor amounts of dimeric products derived from the base-induced reaction of 4-nitrobenzyl chloride mainly *trans*-4,4'-dinitrostilbene (\approx 1%) and 4-nitrotoluene were obtained. Some minor polar products (not characterized) are likely to be a complex mixture of oxidation-reduction products involving the chromanyl moieties of α -T₃ and α -T or the nitro-group of 4-nitrobenzyl chloride.

Characterization of the ether cross products

The major cross products obtained in the reactions of 4-nitrobenzyl chloride with α -T₃ and α -T are yellowish liquids which have molecular weights of 559.59 and 565.31, respectively, suggesting of compounds consisting of one nitrobenzyl and one α -T₃ or α -T fragment. Generally, small changes in the chemical shifts of ¹³C NMR resonance peaks are observed for these products as compared to their starting materials, i.e. α -T₃, α -T and

Table 2.1 Characteristics of products* from the reactions of α -T₃ and α -T with 4-nitrobenzyl chloride

α -T ₃	1a	2a	3a	α -T	1b	2b	3b	4	5	6
Yield (% wt.)	-	70.0	2.2	1.9	-	72.0	2.1	1.9	0.4	1.0
m/z (recorded)	424.4	559.59	559.58	559.58	430.4	565.31	565.4127 [#]	565.4112 [#]	565.40	599.32
m/z (calculated)	424.33	559.37	559.37	559.37	430.38	565.41	565.4131	565.4131	565.41	599.37
R _f TLC ^a	0.19	0.37	0.12	0.12	0.24	0.43	0.14	0.14	0.14	0.60
HPLC r.t. (min) ^b	11.0	9.6	19.6	23.2	9.8	8.5	18.5	22.0	15.8	6.0
Ratio ^c	-	-	53%	47%	-	-	54%	46%	-	-

* Structures of the products are shown in Fig. 2.1.

[#] The exact masses were measured by high resolution mass spectrometry.^a Solvent for silica gel TLC is 5% ethyl acetate in hexanes.^b Retention time for normal phase HPLC using a 4.6 x 250 mm 5 μ m silica column, UV detector at λ_{max} 254 nm, eluent was 2% ethyl acetate in *n*-hexane with a flow rate of 1.5 mL/min.^c Ratios for the diastereomeric compounds 2a:3a and 2b:3b were determined from their ¹H NMR data.

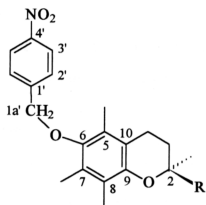
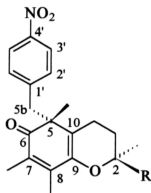
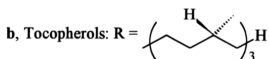
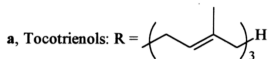
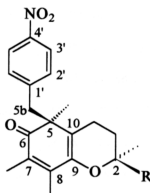
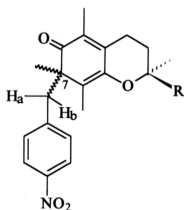
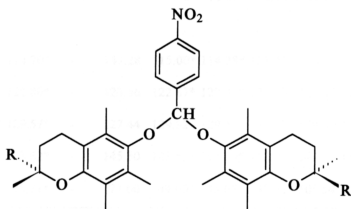
**1a or 1b****2a or 2b (5R)****3a or 3b (5S)****4****5**Fig. 2.1 Structures of the cross products from 4-nitrobenzyl chloride and α -vitamers.

Table 2.2 ^{13}C NMR data and assignments for the reaction products[#] of vitamin E and 4-nitrobenzyl chloride

Carbon No.	$\alpha\text{-T}_3$	1a	2a (R-)	3a (S-)	$\alpha\text{-T}$	1b	2b (R-)	3b (S-)	4	5	4-nitrobenzyl chloride
<i>in chromanyl</i>											
10	122.51	127.56	146.45	146.47	122.66	127.55	146.40*	146.47*	146.45	128.31	-
9	145.94	147.70	148.18*	148.12*	145.58	147.66	148.24*	148.21*	145.48	145.18	-
8	121.26	125.64	146.05	145.73	121.10	125.64	146.05*	145.87*	144.99	126.77	-
7	118.69	123.12	146.05	145.73	118.56	123.10	146.05*	145.87*	53.36	126.64	-
6	144.54	148.10	203.35	203.32	144.56	148.16	203.35	203.37	203.18	148.19	-
5	117.17	117.65	51.88*	51.92*	117.39	117.69	51.87	51.88	127.76	117.74	-
4	20.75	20.60	22.68	22.68	20.81	20.64	22.64	22.65	22.69	20.71	-
3	31.63	31.27	31.01	30.90	31.59	31.18	30.98	30.98	31.39	31.49	-
2	74.20	74.60	75.14	75.12	74.56	74.83	75.34	75.34	75.99	74.82	-
8a	11.79	11.87	11.27*	11.18*	11.83	11.90	11.26*	11.21*	11.57	12.27	-
7a	11.79	12.74	14.25*	14.17*	12.27	12.75	14.23*	14.14*	19.56	12.30	-
5a	11.32	11.76	18.82	18.86	11.35	11.79	18.82*	18.86*	10.10	11.79	-
<i>in nitrobenzyl</i>											
1'	-	147.29	115.02*	114.70*	-	147.28	115.00*	114.78*	123.82	148.24	144.31
2',6'	-	123.54	122.90*	122.80*	-	123.56	122.87*	122.83*	122.50	123.05	123.85
3',5'	-	127.44	129.40*	129.57*	-	127.44	129.52	129.54	129.72	128.40	129.29
4'	-	145.47	143.56*	143.62*	-	145.50	143.53*	143.59*	142.99	144.54	147.63
Ar-CH ₂ or -CH	-	72.96	44.61*	45.21*	-	73.00	44.60*	45.07*	45.92	105.54	44.48

[#] Structures are shown in Fig. 2.1.

* Distinguishable peaks with a difference in chemical shift more than 0.05 ppm were observed when ^{13}C NMR spectra for a mixture of the *R*- and *S*-diastereomers were recorded on a high resolution spectrometer.

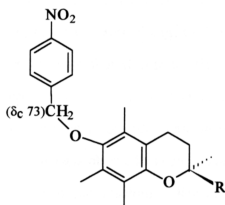
Table 2.3 ^1H NMR data and assignments for the reaction products[#] of vitamin E and 4-nitrobenzyl chloride

Proton	$\alpha\text{-T}_3$	1a	2a (R-)	3a (S-)	$\alpha\text{-T}$	1b	2b (R-)	3b (S-)	4	5	4-nitrobenzyl chloride
<i>in chromanyl moiety</i>											
6-OH	4.21	-	-	-	4.21	-	-	-	-	-	-
4-CH ₂ (H _X)	2.69	2.63	2.05	2.25	2.65	2.65	2.06	2.25	2.35	2.49	-
4-CH ₂ (H _Y)	2.69	2.63	2.25	2.10	2.65	2.65	2.24	2.10	2.15	2.49	-
5a-CH ₃	2.19	2.21	1.32	1.33	2.14	2.24	1.32	1.32	1.91	2.03	-
7a-CH ₃	2.19	2.16	1.86	1.80	2.10	2.20	1.85	1.81	1.60	1.81	-
8a-CH ₃	2.19	2.14	1.79	1.74	2.10	2.17	1.79	1.76	1.66	1.78	-
3-CH ₂ (H _C)	1.90	1.90	1.60	1.65	1.90	1.90	1.60	1.65	1.80	1.4-1.6	-
3-CH ₂ (H _D)	1.90	1.90	1.7	1.65	1.90	1.90	1.70	1.65	1.80	1.4-1.6	-
2a-CH ₃	1.30	1.31	1.02	1.15	1.33	1.34	1.00	1.15	1.33	1.34	-
<i>in benzyl moiety</i>											
Ar(3",5")-H	-	8.29	8.00	7.97	-	8.28	8.00	8.00	7.98	8.17	8.26
	-	8.26	7.97	7.94	-	8.25	7.97	7.97	7.94	8.14	8.16
Ar(2",6")-H	-	7.69	7.03	6.98	-	7.71	7.02	7.00	7.03	7.55	7.62
	-	7.66	6.99	6.95	-	7.68	6.99	6.97	6.99	7.52	7.52
Ar-CH ₂ - (H _a)	-	4.83	3.45	3.32	-	4.85	3.45	3.38	3.25	5.69 [#]	4.66
	-	-	3.39	3.27	-	-	3.39	3.33	3.21	-	-
Ar-CH ₂ - (H _b)	-	4.83	2.92	2.90	-	4.85	2.91	2.91	2.97	-	4.66
	-	-	2.86	2.84	-	-	2.86	2.86	2.93	-	-

* Structures are shown in Fig. 2.1.

[#] Chemical shift for ArCHCl₂.

4-nitrobenzyl chloride (see Table 2.2). The resonance for the methylene carbon of the 4-nitrobenzyl group is shifted from δ_C 44.48 ppm in 4-nitrobenzyl chloride to δ_C 72.96 ppm for **1a** and 73.00 ppm for **1b**, suggesting of a $-\text{CH}_2-\text{O}-$ group. ^1H NMR peaks for the 4-nitrobenzyl, tocopherol and tocotrienol entities are only slightly altered in the ether cross products (Table 2.3). The chemical shifts for the benzylic methylene protons ($\text{Ar}-\text{CH}_2-$) are slightly shifted from δ_H 4.66 ppm (in 4-nitrobenzyl chloride) to 4.83 ppm for **1a** and 4.85 ppm for **1b**.

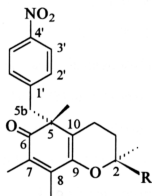
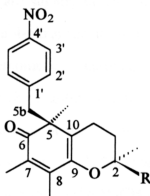


1a and 1b

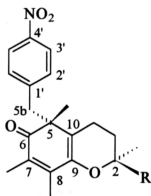
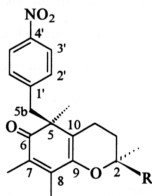
Characterization of carbon-carbon cross products

A yellowish liquid fraction from the reaction mixture obtained by normal phase column chromatography was found to be cross products of 4-nitrobenzyl radical and radicals from $\alpha\text{-T}_3$ or $\alpha\text{-T}$. ^1H and ^{13}C NMR data indicate that these actually were mixtures consisting of isomeric pairs but having similar TLC R_f values. Further purification of these isomers was by normal phase HPLC as they have different retention times (refer to Table 2.1). Mass spectral data indicate that they are cross products of 4-nitrobenzyl and $\alpha\text{-T}_3$ or $\alpha\text{-T}$ radicals with the expected molecular weights of 559.37 and 565.41 from the reactions of 4-nitrobenzyl chloride with $\alpha\text{-T}_3$ and $\alpha\text{-T}$, respectively (Table 2.1), the difference in mass arising from the saturated side chain in $\alpha\text{-T}$ and the triple unsaturation of $\alpha\text{-T}_3$. Structures of these diastereomeric products characterized as

5*R*- and 5*S*-(4-nitrobenzyl)- α -tocotrienone (**2a** and **3a**) and 5*R*- and 5*S*-(4-nitrobenzyl)- α -tocopherone (**2b** and **3b**). From their ^{13}C NMR spectral data (Table 2.2), two aromatic (doublet) resonances (i.e. δ_{C} 122.90 and 129.40 ppm for **2a**, 122.80 and 129.57 ppm for **3a**, 122.87 and 129.52 ppm for **2b**, 122.83 and 129.54 ppm for **3b**), have intensities approximately twice those of other peaks, therefore are assigned to the 4-nitroaromatic carbon pairs C-2" & C-6" and C-3" & C-5", respectively. A resonance peak at δ_{C} 203.3 ppm indicating a carbonyl group (at C-6 in the α -T₃ and α -T moieties) is present in all of the diastereomeric products. For carbons derived from chromanyl moieties of α -T₃ and α -T, i.e. C-7, C-8 and C-10, relatively large changes of their original chemical shifts were observed, viz. from δ_{C} 118-122 ppm shifted to lower field (δ_{C} 145-146 ppm) for the cross products. It means that aromaticity is no more retained in the chromanyl moieties; instead, olefinic carbons are now present in the products. In addition, the aromatic C-5 carbon in α -T₃ and α -T (δ_{C} 117 ppm) has obviously been converted to a saturated carbon with resonance at δ_{C} 51.9 ppm, and the resulting adjacent C-5a methyl carbon had resonance at lower field (δ_{C} 18.8 ppm) as compared to the methyl (δ_{C} 11-12 ppm) bonded to aromatic carbon in the α -vitamers. Chemical shifts for other carbons including C-2, C-3, C-4 and carbons in the side chains of α -T₃ and α -T are unaffected or only slightly altered in the carbon-carbon cross products.

**2a or 2b****3a or 3b**

5*R*- and 5*S*-(4-nitrobenzyl)- α -tocotrienone (**2a** and **3a**) and 5*R*- and 5*S*-(4-nitrobenzyl)- α -tocopherone (**2b** and **3b**). From their ^{13}C NMR spectral data (Table 2.2), two aromatic (doublet) resonances (i.e. δ_{C} 122.90 and 129.40 ppm for **2a**, 122.80 and 129.57 ppm for **3a**, 122.87 and 129.52 ppm for **2b**, 122.83 and 129.54 ppm for **3b**), have intensities approximately twice those of other peaks, therefore are assigned to the 4-nitroaromatic carbon pairs C-2" & C-6" and C-3" & C-5", respectively. A resonance peak at δ_{C} 203.3 ppm indicating a carbonyl group (at C-6 in the α -T₃ and α -T moieties) is present in all of the diastereomeric products. For carbons derived from chromanyl moieties of α -T₃ and α -T, i.e. C-7, C-8 and C-10, relatively large changes of their original chemical shifts were observed, viz. from δ_{C} 118-122 ppm shifted to lower field (δ_{C} 145-146 ppm) for the cross products. It means that aromaticity is no more retained in the chromanyl moieties; instead, olefinic carbons are now present in the products. In addition, the aromatic C-5 carbon in α -T₃ and α -T (δ_{C} 117 ppm) has obviously been converted to a saturated carbon with resonance at δ_{C} 51.9 ppm, and the resulting adjacent C-5a methyl carbon had resonance at lower field (δ_{C} 18.8 ppm) as compared to the methyl (δ_{C} 11-12 ppm) bonded to aromatic carbon in the α -vitamers. Chemical shifts for other carbons including C-2, C-3, C-4 and carbons in the side chains of α -T₃ and α -T are unaffected or only slightly altered in the carbon-carbon cross products.

**2a or 2b****3a or 3b**

Stereochemistry of the diastereomeric carbon-carbon products

The ^1H NMR data provide some basic information to determine the stereochemistry of the diastereomeric carbon-carbon cross products, **2a** & **3a** and **2b** & **3b**. The aromatic protons at position C-2", C-3", C-5" and C-6" of the 4-nitrobenzyl moiety in the carbon-carbon cross products remained unsubstituted but these para-substituted doublet peaks are slightly shifted to upfield (see Table 2.3). The chemical shifts for the olefinic methyl protons at C-7a and C-8a (δ_{H} 2.1-2.2 ppm in $\alpha\text{-T}_3$ and $\alpha\text{-T}$) are shifted to upfield δ_{H} 1.7-1.9 ppm; resonances of the protons of methyl C-5a attached to a saturated carbon (C-5) is shifted further upfield to δ_{H} 1.32 ppm. Significant differences are noted in the carbon chemical shifts of the 2a-CH₃ for **2a** (23.59 ppm) and **3a** (23.10 ppm) as well as the **2b** (23.60 ppm) and **3b** (23.27 ppm). Large differences in the proton chemical shifts of the axial 2a-CH₃ are also observed for the diastereomeric pairs, *viz.* 1.02 ppm for **2a**, 1.15 ppm for **3a**, 1.00 ppm for **2b** and 1.15 ppm for **3b**; these results indicate that the 4-nitrophenyl group attached to the axial carbon-5b can exert an appreciable anisotropic effect on the axial 2a-CH₃ in the (*R*)-diastereomers (i.e. **2a** & **2b**). Similarly, the 4-nitrophenyl group also causes significant upfield shifts for the protons 4-CH_X (δ_{H} 2.05 ppm) and 3-CH_C (δ_{H} 1.60 ppm) of the (*R*)-diastereomers. In the (*S*)-diastereomers (i.e. **3a** & **3b**), upfield shifts of the protons 4-CH_Y (δ_{H} 2.10 ppm) and 3-CH_D (δ_{H} 1.65 ppm) are due to the anisotropic effect from the 4-nitrophenyl group; resonance peaks for 7a-CH₃ and 8a-CH₃ (δ_{H} 1.86 & 1.79 ppm for **2a**) are slightly downfield as compared to δ_{H} 1.80 & 1.74 ppm in the (*S*)-diastereomer. The 4-nitrobenzylic methylene protons (5b-CH₂) are also distinguishable among the diastereomeric structures, and molecular simulation data (Table 2.4) may be useful to explain these differences that the 5b-CH₂-group is axial in the (*R*)-diastereomer but it is equatorial in the (*S*)-diastereomer. The chemical shifts for 5b-CH_a-proton in the (*R*)-diastereomers **2a** & **2b** (both δ_{H} 3.45 & 3.39 ppm, J_{ab} 14.16 Hz) are relatively downfield as compared to the 5b-CH_a-proton in **3a** (3.32 & 3.27 ppm, J_{ab} 14.16 Hz) and **3b** (3.38 & 3.33 ppm, J_{ab} 14.16 Hz) which are in

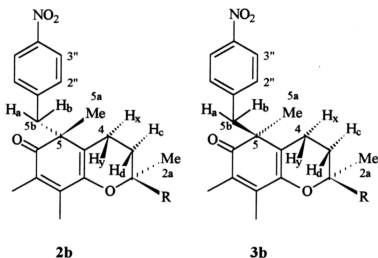


Table 2.4 NMR and molecular simulation* data for the diastereomeric carbon-carbon cross products from α -T and 4-nitrobenzyl chloride

	2b	3b
Configuration at C-5	<i>R</i>	<i>S</i>
<u>NMR data (ppm)</u>		
δ_C 5b-CH ₂	44.60	45.07
δ_H 5b-H _a (doublet)	3.45, 3.39	3.38, 3.33
δ_H 5b-H _b (doublet)	2.91, 2.86	2.91, 2.86
δ_C 5a-CH ₃	18.82	18.86
δ_H 5a-CH ₃	1.32	1.32
δ_C 2a-CH ₃	23.60	23.27
δ_H 2a-CH ₃	1.00	1.15
<u>Energy-optimized data*</u>		
5a-CH ₃	equatorial	axial
5b-CH ₂	axial	equatorial
6-C=O to 5b-CH ₂	3.29 Å	2.78 Å
6-C=O to 5b-CH _a	3.69 Å	2.67 Å
6-C=O to 5b-CH _b	4.26 Å	3.89 Å
6-C=O to 5a-CH ₃	2.73 Å	3.33 Å

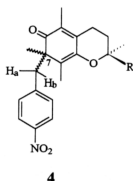
* Energy-optimized structures were simulated by Insight II version 2.3.5 (Biosym).

S-configuration; this is apparently due to a relatively strong deshielding effect from the 6-C=O exerts on the axial methylene group in the (*R*)-diastereomers. However, such an effect does not apply to 5b-H_b-proton possibly because of its orientation being more hindered and longer distance from the 6-C=O. 2D-NMR techniques were applied to confirm the absolute configurations of the diastereomeric structures. NOESY spectra of the diastereomeric compounds **2b** and **3b** are shown in Fig. 2.2. The 5b-H_a and 5b-H_b protons show strong NOE interactions with adjacent 5a-CH₃ in both diastereomers, but only 5b-H_b of the (*S*)-diastereomer **3b** shows strong NOE interaction with 4-H_y. For the (*R*)-diastereomer **2b**, the expected NOE enhancement between 4-H_y and 5a-CH₃ is observed. However, the 4-H_x proton in the (*S*)-diastereomer **3b**, shows strong NOE interactions with 5a-CH₃, 3-H_c and 2a-CH₃, and strong NOE effect between the 5a-CH₃ with 3-H_c is also observed. The major NOE interactions used to assign the absolute configuration of the diastereomeric structures are indicated in Fig. 2.2.

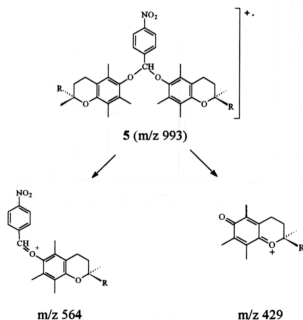
Minor products from the reaction of α-T with 4-nitrobenzyl chloride

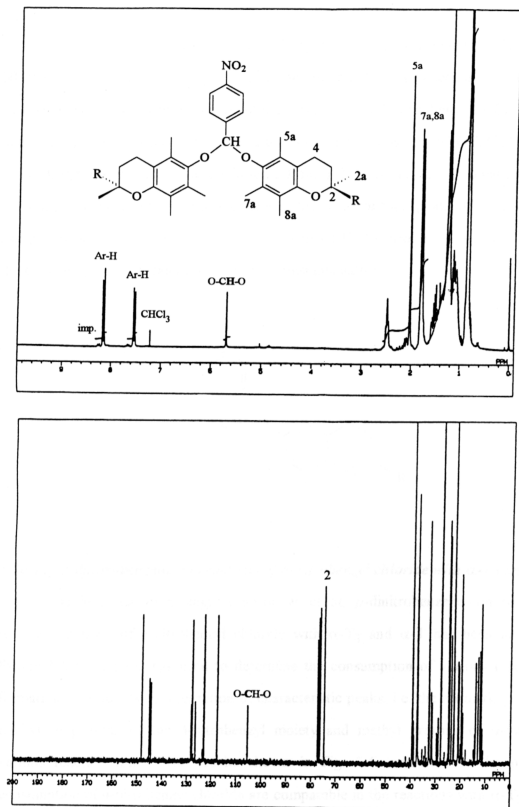
A minor product **4** (with a yield of 0.4%) has been isolated from the reaction of α-T and 4-nitrobenzyl chloride in base. Its ¹³C NMR profile is basically similar to that of **3b**. Comparing with the ¹H NMR data of **3b** (see Table 2.3), **4** has δ_H (3.25 & 3.21 ppm) for the Ar-CH_a- proton at slightly higher field but its δ_H (2.97 & 2.93 ppm) for Ar-CH_b- proton is slightly down-field; and the coupling constant (*J* = 13.2 Hz) is also appreciably smaller as compared to that in **3b** (*J* = 13.7 Hz). Compound **4** characterized as 7-(4-nitrobenzyl)-α-tocopherone is an isomer of **3b** with the 4-nitrobenzyl group bonded to the chromanyl moiety at C-7.

Another minor product **5** (yield ≈ 1%) obtained from the reaction of α-T with 4-nitrobenzyl chloride in base is a non-polar yellow liquid. Mass spectroscopic data indicate that **5** has a molecular weight of 993, the molecular ion (*m/z* 993, 1%) cleaves to give more stable fragments α-TOCHAR⁺ (*m/z* 564, 46%) and α-TO⁺ (*m/z* 429, 57%). ¹³C and

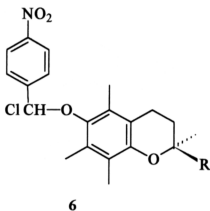


^1H NMR spectral data are given in Tables 2.2 and 2.3, respectively, the ^1H and ^{13}C NMR spectra of the acetal cross product **5** are shown in Fig. 2.3. It is clear that the four aromatic carbons in the 4-nitrobenzyl group remained intact; one proton resonance at δ_{H} 5.69 ppm is assigned to a methine proton. As compared to α -T (Table 2.3), the aromatic methyl protons of C-5a, C-7a and C-8a of the chromanyles are slightly shifted upfield while other protons in the α -tocopheroxy moieties remained unchanged. For ^{13}C NMR assignments, chemical shifts for most of the carbons in α -tocopheroxy moieties are only slightly different from those in α -T; the resonance peak at δ_{C} 105.54 ppm is due to the methine carbon which bears one 4-nitrophenyl group and two α -tocopheroxy moieties, and it is confirmed to be a methine carbon (doublet) by using off-resonance NMR technique.



Fig. 2.3 ^1H and ^{13}C NMR spectra of 5.

A trace amount of a yellowish liquid product has been isolated by HPLC, its ^1H NMR spectrum is shown in Fig. 2.4. The compound (6) has a molecular ion peak at m/z 599 which corresponds to a compound consisting of one 4-nitrophenyl- CHCl - group and one α -tocopheroxy moiety. Cleavage of the chlorine gives rise to $\alpha\text{-TOCHAr}^+$ (m/z 564) and further fragmentation gives the base peak for $\alpha\text{-TO}^+$ at m/z 429. Aromatic protons of its 4-nitrophenyl group remained unsubstituted, a resonance peak at δ_{H} 5.14 ppm is assigned to the methine proton in the 4-nitrophenyl- CHCl - group. A low yield (0.1%) of the coupling product 6 can be a result of its unstable nature.



*Effect of *p*-dinitrobenzene on reactions of 4-nitrobenzyl chloride with $\alpha\text{-T}_3$ and $\alpha\text{-T}$*

The influence of a single electron acceptor, *p*-dinitrobenzene, on the electron transfer reactions of 4-nitrobenzyl chloride with $\alpha\text{-T}_3$ and $\alpha\text{-T}$ has been investigated. Proton NMR analysis was used to determine the consumption of various reactants and formation of products by integration of characteristic peaks, i.e. the aromatic protons and methylene protons of the 4-nitrobenzyl moiety and methyl protons of α -vitamers at various reaction times. The results shown in Fig. 2.5 indicate that the profiles of consumption of 4-nitrobenzyl chloride are comparable in the reactions with $\alpha\text{-T}_3$ and $\alpha\text{-T}$ due to their similar chromanyl structures, and similar results were observed when *p*-dinitrobenzene was added. In the absence of *p*-dinitrobenzene, the reactions were completed when all of the 4-nitrobenzyl chloride and α -vitamers were reacted in 5 hours.

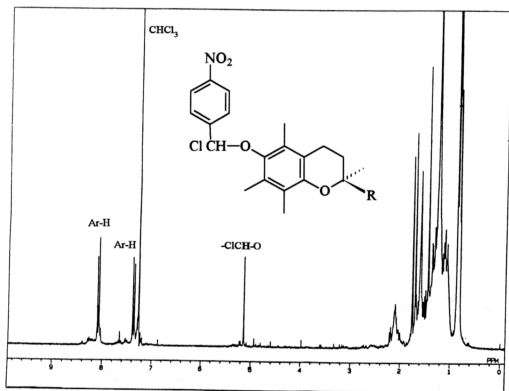


Fig. 2.4 ^1H NMR spectrum of **6**.

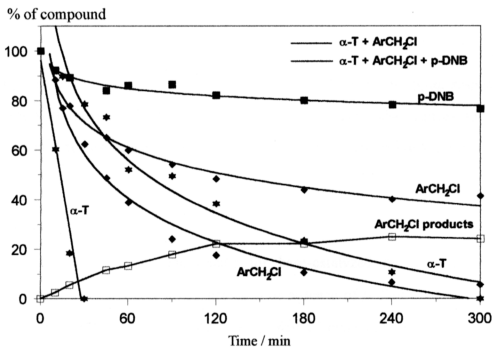
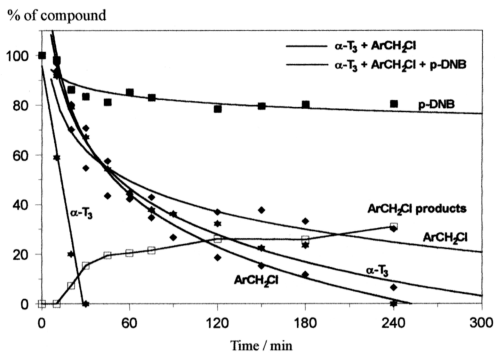


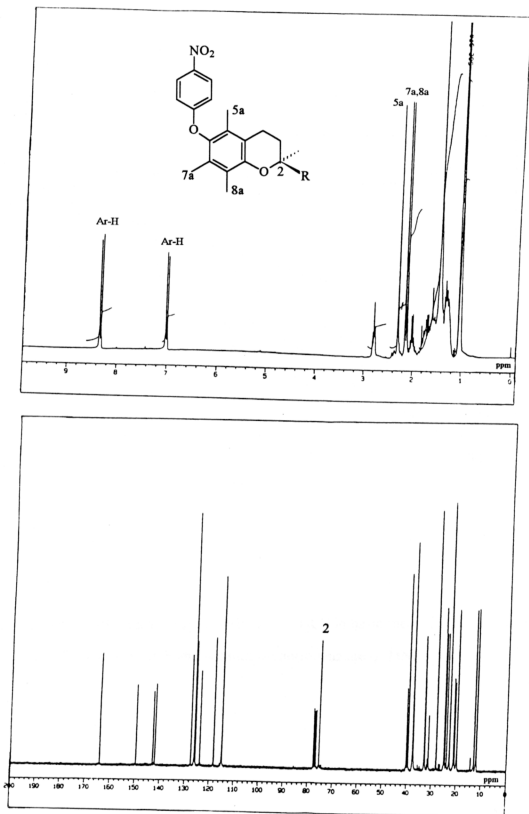
Fig. 2.5 Reactions of 0.1 M 4-nitrobenzyl chloride (ArCH_2Cl) with 0.1 M $\alpha\text{-T}_3$ (top) and $\alpha\text{-T}$ (bottom) in basic medium in the presence or absence of 0.1 M p -dinitrobenzene ($p\text{-DNB}$).

In the presence of *p*-dinitrobenzene, a new reaction seems to occur, all of the α -T₃ and α -T were consumed in approximately 30 min while only a portion (about 30-45%) of the 4-nitrobenzyl chloride reacted.

Reaction of α -T and *p*-dinitrobenzene in basic medium was also carried out to provide qualitative information in order to investigate for a possible role of *p*-dinitrobenzene in the reaction of α -T with 4-nitrobenzyl chloride. The major product purified by column chromatography was a yellow liquid with a yield of 80% (based on the weight of α -T), mass spectrum of the compound with molecular ion at *m/z* 551 indicates that it is an ether cross product consists of one unit of nitrophenyl and one unit of α -T. ¹H and ¹³C NMR spectra of the nucleophilic displacement product *p*-nitrophenyl- α -tocopherol (7) are shown in Fig. 2.6. Aromatic protons in the *p*-nitrophenyl moiety, viz. at C-2" & C-6", give a doublet at δ_H 8.16 ppm and *J* = 9.3 Hz, and protons at C-3" & C-5" give a doublet at δ_H 6.83 ppm and *J* = 9.3 Hz. Substitution of the phenolic proton by the *p*-nitrophenyl has resulted an upfield shift of the resonance peaks for C-5a, C-7a and C-8a methyl protons in the chromanyl ring to δ_H 2.12, 1.98 and 1.94 ppm, respectively. The electron-withdrawing effect by the *p*-nitrophenyl moiety also causes the chromanyl carbons in 7 to resonance at lower field, whereas the other carbons are about similar to those in α -T.

Kinetic results

The preliminary kinetic results for base-catalyzed reactions of 4-nitrobenzyl chloride with α -T₃ and α -T in the presence or absence of *p*-dinitrobenzene are shown in Fig. 2.7. Gradients of the plots for the initial (first 60 min) disappearance of 4-nitrobenzyl chloride are comparable for the reactions with α -T₃ ($k_1 = 1.46 \times 10^{-2} \text{ min}^{-1}$, $r^2 = 0.98$) and α -T ($k_1 = 1.57 \times 10^{-2} \text{ min}^{-1}$, $r^2 = 0.99$). In the presence of *p*-dinitrobenzene, the initial rates for reactions of 4-nitrobenzyl chloride with α -T₃ ($k_1 = 1.56 \times 10^{-2} \text{ min}^{-1}$, $r^2 = 0.91$) and α -T ($k_1 = 8.4 \times 10^{-3} \text{ min}^{-1}$, $r^2 = 0.98$) were also within a comparable range as for the reactions without *p*-dinitrobenzene; however, when all of the α -T₃ and α -T have been

Fig. 2.6 ^1H and ^{13}C NMR spectra of 7.

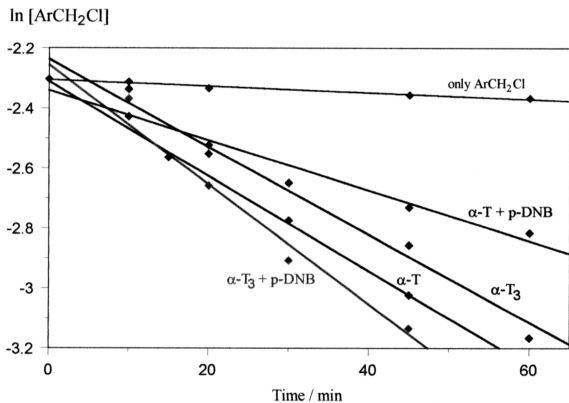
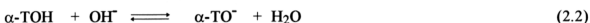
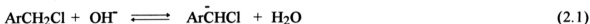
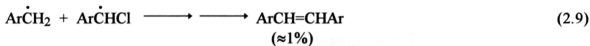
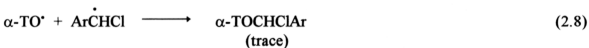
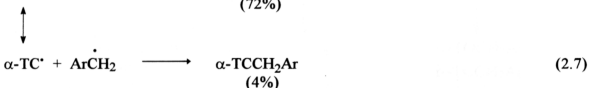
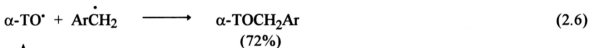


Fig. 2.7 Reactions of 4-nitrobenzyl chloride (ArCH_2Cl) in basic media in the presence of α -T₃ and α -T, with and without *p*-dinitrobenzene (*p*-DNB).

reacted after 60 min, these gradients were drastically reduced to $k_1 = 2.15 \times 10^{-3} \text{ min}^{-1}$ (for reaction with $\alpha\text{-T}_3$, $r^2 = 0.96$) and $k_1 = 1.57 \times 10^{-3} \text{ min}^{-1}$ (for reaction with $\alpha\text{-T}$, $r^2 = 0.85$). It means once all of the α -vitamers reacted, the consumption of 4-nitrobenzyl chloride was slowed down to a rate comparable to that for reaction of 4-nitrobenzyl chloride with base (initial rate $k_1 = 3.1 \times 10^{-4} \text{ min}^{-1}$, $r^2 = 0.92$). Although the kinetic data have not used optimized concentrations, the results suggest a possible role of α -vitamers as electron donors in the electron transfer reactions involving good electron acceptors.

Mechanism for the reaction of 4-nitrobenzyl chloride with $\alpha\text{-T}$ or $\alpha\text{-T}_3$

Present product studies suggest that the reaction of 4-nitrobenzyl chloride with $\alpha\text{-T}$ under a basic condition may undergo a series of complex reactions providing evidence of radical derived products. The possible pathways are exemplified in Scheme 2.6, the major steps of the radical mechanism are also diagrammatically illustrated in Fig. 2.8. A similar mechanism is also applicable to the reaction when $\alpha\text{-T}$ is replaced by $\alpha\text{-T}_3$ which has the same chromanyl structure as $\alpha\text{-T}$. Formation of the radical products suggest that a single electron transfer step has taken place in the reaction of 4-nitrobenzyl chloride with $\alpha\text{-T}$ to produce the α -tocopheroxyl radical ($\alpha\text{-TO}^\bullet$). Although $\alpha\text{-TO}^\bullet$ radicals can be generated directly through H-abstraction mechanism, the lack of major amounts of *p*-nitrotoluene favours the electron-transfer pathway although a simple S_N2 reaction cannot be excluded. Among the various resonance structures of radicals derived from $\alpha\text{-T}$, $\alpha\text{-TO}^\bullet$ radical is the most stable based on the ESR spin densities (see Chapter 1).

Scheme 2.6 Mechanism for the Reaction of 4-Nitrobenzyl Chloride with α -T**Initiation and Propagation**Termination*

* α -TOH is α -tocopherol (= α -T), ArCH_2Cl is 4-nitrobenzyl chloride. The yields given in bracket for α -T cross products are based on the weight of α -T, whereas the yield for dinitrostilbene is based on 4-nitrobenzyl chloride.

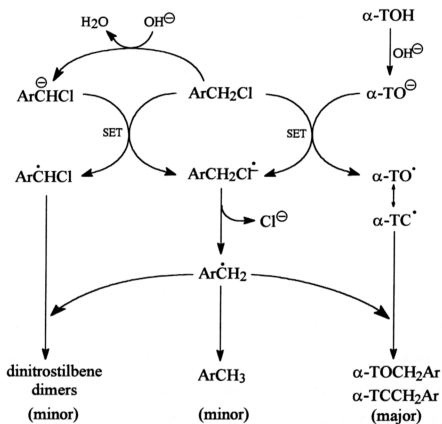
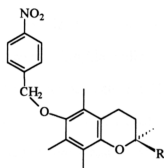


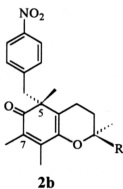
Fig. 2.8 Postulated radical mechanism for the reaction of α -T and 4-nitrobenzyl chloride with base.

The reaction of 4-nitrobenzyl chloride with α -T afforded several types of cross-products as shown in Fig. 2.1. The major product is an ether compound α -TOCH₂Ar (i.e. **1b**) which is expected to be formed by cross coupling of 4-nitrobenzyl radical (ArCH₂·) with the α -TO· (Equation 2.6) but would be indistinguishable from a S_N2 reaction products.

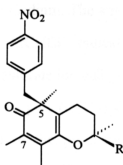


1b (α -TOCH₂Ar)

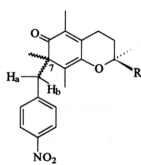
In the present reactions, carbon-carbon cross products α -TCCH₂Ar (i.e. **2b** and **3b**) are found to be substantial major products and are apparently the result from coupling of the 4-nitrobenzyl radical and α -tocopheroxyl radical as described by Equation 2.7. It is noted that the products formed by cross coupling of the 4-nitrobenzyl radical at the C-5 of α -T have higher yields (for **2b** and **3b**) than that coupling at C-7 (for **4**), this may be attributed to a radical stabilization induced by the pyran ring. Carbon-carbon coupling at C-5 of α -T gives rise to diastereomeric products having (*R*)- and (*S*)-configurations (i.e. **2b** and **3b**, respectively) with a ratio of approximately 1:1 (see Table 2.1), results indicating random radical coupling.



2b



3b

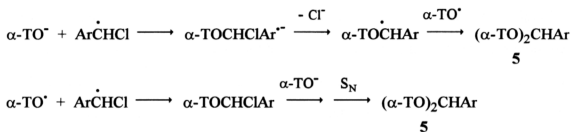


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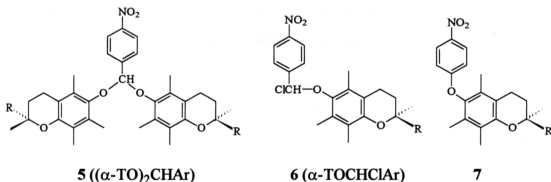
Under a strong basic condition, abstractions of the acidic α -proton from 4-nitrobenzyl chloride and the phenolic proton from α -T by hydroxide ion can occur readily and give rise to the respective anions (Equations 2.1 & 2.2). There is a competing reaction as well and is expected to follow the well known electron transfer process [106,107,216,316], the α -chloro-4-nitrobenzyl carbanion will transfer an electron to another 4-nitrobenzyl chloride molecule (Equation 2.4) thus form α -chloro-4-nitrobenzyl radical (ArCHCl^\bullet) and 4-nitrobenzyl chloride radical anion ($\text{ArCH}_2\text{Cl}^{\bullet-}$). This electron transfer step is slower than that of reaction with α -T (Equation 2.3). The radicals derived from 4-nitrobenzyl chloride, i.e. ArCH_2^\bullet and ArCHCl^\bullet should lead to the radical products such as $\text{ArCH}=\text{CHAr}$ (or dinitrostilbene) and α -TOCHClAr (6) are however only in relatively low yields. Moreover, the present crude kinetic experiments show that the disappearance of 4-nitrobenzyl chloride in the basic phase transfer reaction was very slow, but the rate of the consumption was drastically increased when α -T was added. Electron transfer from the α -tocopheroxyl anion ($\alpha\text{-TO}^{\bullet-}$) to the ArCH_2Cl (Equation 2.3) appeared to be the dominant route for the generation of $\alpha\text{-TO}^\bullet$ radical and $\text{ArCH}_2\text{Cl}^{\bullet-}$ radical anion. The $\text{ArCH}_2\text{Cl}^{\bullet-}$ radical anion is an unstable intermediate and readily converted to 4-nitrobenzyl radical (ArCH_2^\bullet) by loss of a chloride ion (Equation 2.5). The yield of $\alpha\text{-TOCH}_2\text{Ar}$ (1b) is high (72% based on the weight of α -T), indicates a preferential C-O coupling in the absence of $\text{S}_{\text{N}}2$ competitive reaction.

In order to explain the formation of the small amount (1%) of an acetal cross product ($\alpha\text{-TO}$)₂CHAr (5), a minor pathway may also occur simultaneously during the reaction of α -T with ArCH_2Cl in basic medium. The key step may be the combination of α -T anion with a portion of the ArCHCl^\bullet radicals to produce a radical anion ($\alpha\text{-TOCHClAr}^{\bullet-}$) which upon losing a chloride ion will give rise to a radical ($\alpha\text{-TOCHAR}^\bullet$) and subsequently couples with $\alpha\text{-TO}^\bullet$ to give ($\alpha\text{-TO}$)₂CHAr; a hydrogen-abstraction by the $\alpha\text{-TOCHAR}^\bullet$ may also contribute to the production of $\alpha\text{-TOCH}_2\text{Ar}$ (1b). In an alternative route, coupling of $\alpha\text{-TO}^\bullet$ with ArCHCl^\bullet leads to $\alpha\text{-TOCHClAr}$ (6) which is

unstable and may undergo S_N reactions to yield **5**. However, these postulated pathways based on product analyses are yet to be confirmed by detection of the radical and radical anion.



Significant amounts of carbon-carbon radical products obtained in the present studies provide evidence to support radical mechanisms in which 4-nitrobenzyl chloride can act as an electron acceptor [106] while the α -tocopherol anion acts as an electron donor. The electron transfer mechanism for the reaction of 4-nitrobenzyl chloride with base is supported by kinetic evidence [107,216]. The present preliminary kinetic data indicate that addition of α -T drastically increase the consumption of 4-nitrobenzyl chloride (Fig. 2.7). In another experiment, addition of a good electron acceptor, *viz.* *p*-dinitrobenzene, accelerated the consumption of α -T but reduced the consumption of 4-nitrobenzyl chloride (Fig. 2.5). However, this is due to the diversion of reaction of α -T with *p*-dinitrobenzene to give rise to *p*-nitrophenyl- α -tocopherol ether (**7**).



2.3.2 Reaction of α -T with Triphenylmethyl Chloride

To further investigate the electron transfer reaction of α -T, another system using a common electron acceptor namely triphenylmethyl chloride has been carried out. When triphenylmethyl chloride in dichloromethane was mixed with aqueous NaOH in the presence of a phase transfer catalyst (i.e. benzyltriethyl ammonium chloride), triphenyl methanol has been obtained as the sole product. However, when triphenylmethyl chloride and α -T were mixed together with base, the reaction was completed in two hours and a mixture of products were obtained; some of their characteristic data are given in Table 2.5. The reaction of α -T gave rise to radical products such as trimers (24%) and dimers (21%) mainly spirodienone dimers, and a mixture of oxidized or partially decomposed products; characterization by ^1H and ^{13}C NMR spectral data showed that the structures of trimers and dimers are similar to those obtained in the oxidation of α -T by alkaline $\text{K}_3\text{Fe}(\text{CN})_6$ as described in Chapter 1. About 4% yield (based on α -T) of non-polar products obtained from the reaction are a mixture of hydrocarbons, as indicated by NMR spectroscopy, these compounds are derived from the side chain of α -T.

Purification of the product mixture by column chromatography also afforded a yellow liquid compound (20% yield) which has a molecular weight of 872.7229 (calculated 872.7261) corresponds to a compound consisting two α -T fragments and one methylene group. Mass spectroscopic data indicate that the parent molecular ion (7%) readily cleaves into $\alpha\text{-TOCH}_2^+$ (m/z 443, 100%) and α -T fragment (m/z 429, 52%). This compound is an acetal cross product of α -T and dichloromethane, named as di- α -tocopheryloxymethane (**8**), its ^1H and ^{13}C NMR spectra are shown in Fig. 2.9.

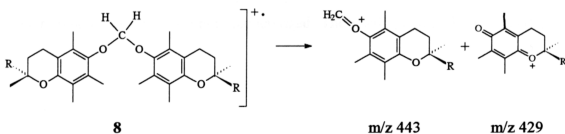


Table 2.5 Characteristics of products from the reaction of α -T with triphenylmethyl chloride

Product	Yield (%)	TLC R_f^a	m/z calculated	m/z recorded
Products of α-T				
non-polar hydrocarbons*	4	0.76	-	-
trimers	24 ^b	0.68	1287.11	1286
dimers	21 ^b	0.20	858.75	859
oxidized products*	18	0.1-0.5	-	-
Reaction with CH_2Cl_2				
di- α -tocopheroxymethane (8) 20 ^b		0.62	872.7261	872.7229 ^c
Products from Ph_3CCl				
Ph_3CH	14 ^d	0.17	-	-
Ph_3COH	77 ^d	0.13	-	-
Benzophenone	2 ^d	0.22	182.07	182.06

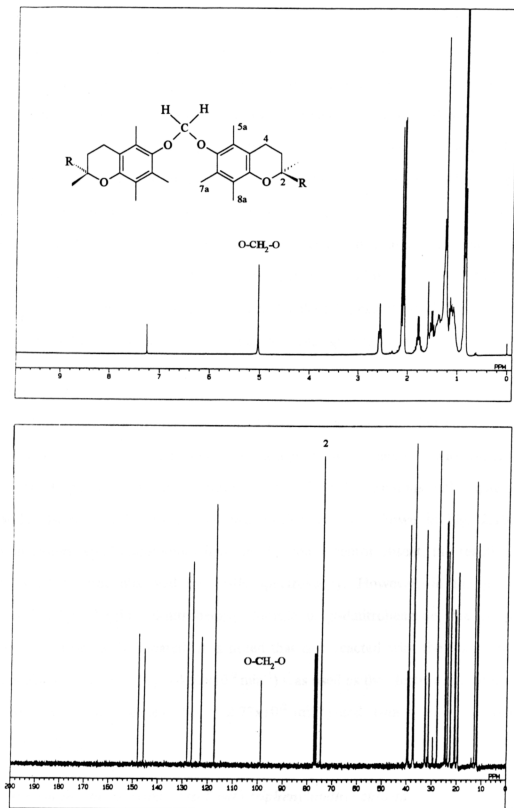
^a Silica gel TLC using 5% ethyl acetate in *n*-hexane.

^b Based on the weight of α -T.

^c Data obtained by high-resolution mass spectrometry.

^d Based on triphenylmethyl chloride.

* Non-polar hydrocarbons were not characterized. Oxidized products are a mixture of polar compounds which are not characterized. The yields presented are relative to α -T reacted.

Fig. 2.9 ^1H and ^{13}C NMR spectra of 8.

Conversion of the free phenolic group (α -TOH) to an ether group in **8** has resulted a small downfield shift for the chromanyl carbons as compared to those in α -T. The presence of a methylenedioxy group ($-\text{O}-\text{CH}_2-\text{O}-$) in **8** which resonances at δ_{H} 5.03 ppm and δ_{C} 99.02 ppm was confirmed as it showed a triplet in OFR NMR spectrum. While α -T reacted rapidly to yield mainly radical products, most of the triphenylmethyl chloride (ca. 77%) reacted to give triphenyl methanol presumably by normal hydrolysis. Only 14% of the triphenylmethyl chloride was found to be converted to triphenylmethane, a product derived from triphenylmethyl radical; its structure was confirmed by ^{13}C NMR and mass spectroscopy. A minor product showed its molecular ion peak at m/z 182.06 was found to be benzophenone as determined by ^1H and ^{13}C NMR spectroscopy.

Kinetic results

The initial rate for the reaction of α -T with triphenylmethyl chloride was also followed by ^1H NMR spectroscopy and compared with reactions with other electron acceptors. Logarithms of the concentrations of α -T in the reactions with triphenylmethyl chloride, 4-nitrobenzyl chloride and *p*-dinitrobenzene are shown in Fig. 2.10. Under present experimental conditions where an electron acceptor absent, the reaction of α -T with NaOH is not observed by NMR spectroscopy. However, in the presence of triphenylmethyl chloride, 4-nitrobenzyl chloride or *p*-dinitrobenzene, the consumption of α -T could be easily initiated. It is noted that α -T reacted with the fastest rate when triphenylmethyl chloride ($k_1 = 4.63 \times 10^{-2} \text{ min}^{-1}$) was used as the electron acceptor, which is followed by *p*-dinitrobenzene ($k_1 = 2.77 \times 10^{-2} \text{ min}^{-1}$) and 4-nitrobenzyl chloride ($k_1 = 1.40 \times 10^{-2} \text{ min}^{-1}$).

Mechanism for the reaction of α -T with triphenylmethyl chloride

In the absence of α -T, triphenylmethyl chloride reacted rapidly with NaOH via S_{N} mechanism to produce triphenyl methanol exclusively. However, when α -T and

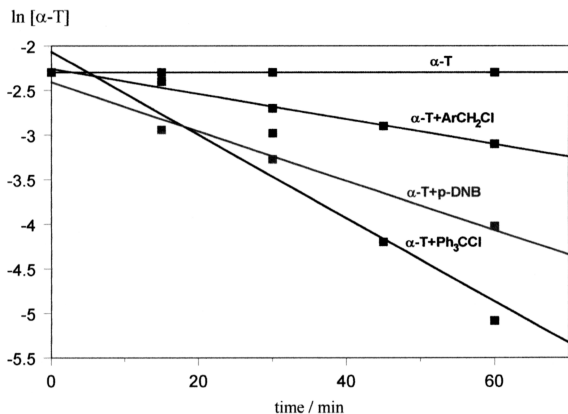
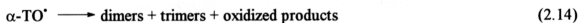
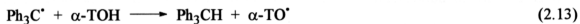
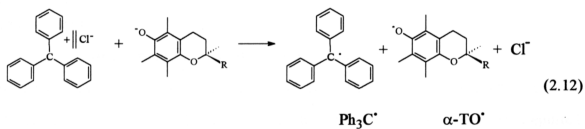
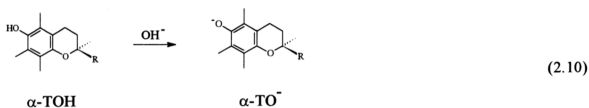


Fig. 2.10 Relative reactivity of $\alpha\text{-T}$ with various electron-accepting agents in basic medium; ArCH_2Cl = 4-nitrobenzyl chloride, $p\text{-DNB}$ = p -dinitrobenzene, Ph_3CCl (triphenylmethyl chloride).

Scheme 2.7 Mechanism for the Reaction of α -T and Triphenylmethyl Chloride

triphenylmethyl chloride mixed together in a basic medium they react *via* competing pathways which may include an electron transfer mechanism as shown in Scheme 2.7. It is clear that there is a rapid dissociation to carbonion ion or its ion pair precursor. An electron transfer to this ion pair also competes to provide the observed products. α -Tocopherol anion (α -TO⁻) could be easily formed under basic conditions (Equation 2.10), single electron transfer from α -TO⁻ to triphenylmethyl cation or ion pair will give rise to α -tocoperoxyl radical and triphenylmethyl radical (Equation 2.12). Coupling of a triphenylmethyl radical to a dimer apparently was not obtained, an indication of a rapid H-transfer from α -T (Equation 2.13). A cross-product of α -tocoperoxyl with triphenylmethyl radical was also not found in this reaction indicative of the presence of undissociated α -T.

The major products derived from the base-catalyzed reaction of α -T and triphenylmethyl chloride are dimers, trimers and other oxidized compounds of α -T (Equation 2.14). The α -T dimers and trimers are similar to those reported for α -tocoperoxyl radicals and are formed *via* radical processes with the triphenylmethyl cation acting as an effective electron acceptor. Radical dimerization of the α -T in this system provides indirect evidence that electron transfer from α -T anion to triphenylmethyl cation or ion-pair has occurred. Another indirect evidence for the formation of triphenylmethyl radical is that triphenylmethane is a major product derived from the triphenylmethyl chloride, it may be formed *via* abstraction of a phenolic hydrogen from α -T (Equation 2.13). An acetal compound derived from α -T and dichloromethane, i.e. di- α -tocoperoxymethane (**8** or $(\alpha$ -TO)₂CH₂), was found to be a major product in the reaction of α -T and triphenylmethyl chloride in dichloromethane; it is a simple S_N product but a lower yield was obtained in the absence of triphenylmethyl chloride.

2.3.3 Reactivity of Various Vitamin E Components

Reactions of γ -vitamers with 4-nitrobenzyl chloride

Ether cross products, i.e. 4-nitrobenzyl- γ -tocopherol ether (9a) and 4-nitrobenzyl- γ -tocotrienol ether (9b), are the major products formed in the base-catalyzed reactions of 4-nitrobenzyl chloride with γ -T and γ -T₃, respectively (Fig. 2.11). The γ -T and γ -T₃ also dimerized in the reactions giving rise to mainly γ -tocopherol dichromanyl ether dimer (10a) and γ -tocotrienol dichromanyl ether dimer (10b), respectively; these dimers are expected to be formed by radical coupling (see Chapter 1). The ether dimers (10a & 10b) further reacted with 4-nitrobenzyl chloride to give ether cross products 11a and 11b, respectively; this reaction was confirmed by reacting pure 10b with 4-nitrobenzyl chloride in basic medium.

Reactivity of vitamin E components

The reactivity of various vitamin E components, i.e. tocopherols (α -T & γ -T), tocotrienols (α -T₃ & γ -T₃) and γ -T dichromanyl ether dimer, in the reactions with 4-nitrobenzyl chloride was also studied. The changes in the logarithms of the initial concentrations of vitamin E were monitored by proton NMR spectroscopy, the results are shown in Fig. 2.12. α -T ($k_1 = 1.08 \times 10^{-2} \text{ min}^{-1}$, $r^2 = 0.98$) and α -T₃ ($k_1 = 1.43 \times 10^{-2} \text{ min}^{-1}$, $r^2 = 0.98$) show comparable reaction rate; the rates for reactions of γ -T ($k_1 = 1.85 \times 10^{-3} \text{ min}^{-1}$, $r^2 = 0.99$) and γ -T₃ ($k_1 = 3.00 \times 10^{-3} \text{ min}^{-1}$, $r^2 = 0.96$) are also fall in the same order. These results indicate that the type of side chain of vitamin E components does not cause a significant effect to the reactivity of the phenolic OH. However, α -T is relatively more reactive than γ -T, and α -T₃ also reacted at a faster rate than γ -T₃. The methyl substitution in the vitamin E moieties substantially influences their reactions with 4-nitrobenzyl chloride; 5,7,8-trimethyl chromanols (i.e. α -T and α -T₃) are apparently more reactive than the 7,8-dimethyl chromanols (i.e. γ -T and γ -T₃). For γ -tocopherol dichromanyl ether dimer (10a) in which a γ -tocopheroxy is substituted at C-5 of another γ -T, reaction rate of the

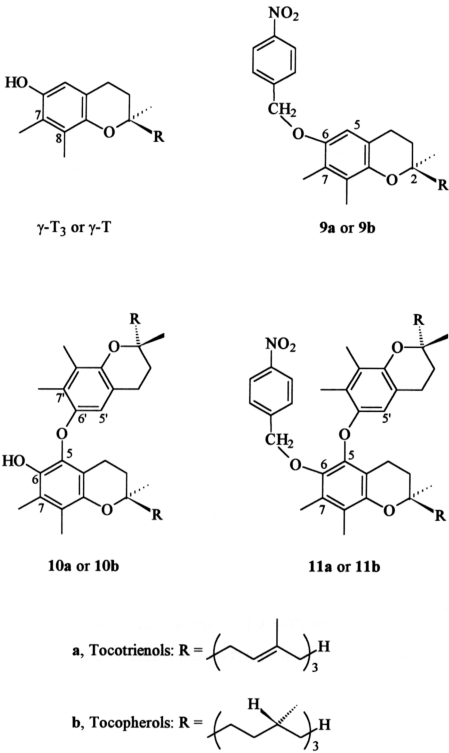


Fig. 2.11 Structures of $\gamma\text{-T}_3$, $\gamma\text{-T}$, their dimers and cross-products with 4-nitrobenzyl chloride.

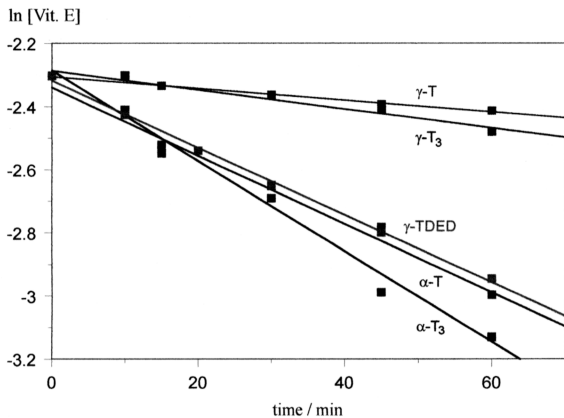
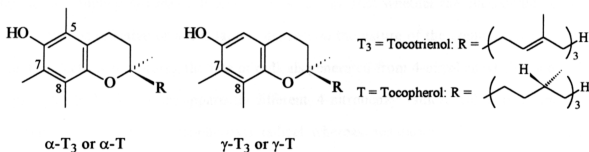


Fig. 2.12 Relative reactivities of tocopherols ($\alpha\text{-T}$ & $\gamma\text{-T}$), tocotrienols ($\alpha\text{-T}_3$ & $\gamma\text{-T}_3$) and $\gamma\text{-T}$ dichromanyl ether dimer ($\gamma\text{-TDED}$ or **10b**) in the reactions with 4-nitrobenzyl chloride.

dimer ($k_1 = 1.06 \times 10^{-2} \text{ min}^{-1}$, $r^2 = 0.99$) is comparable with that of α -T but is relatively faster than for γ -T.

The present results demonstrate that the reactivities of vitamin E components are not significantly affected by the distant side chain, and the reaction centres of the vitamin E components are at the chromanoyl moieties. The electron transfer mechanisms as shown in Scheme 2.6 (p.103) are basically similar for the α -vitamers and γ -vitamers in the reactions with 4-nitrobenzyl chloride, but the number of methyl group substitution in the chromanoyl rings determine their reactivities and affect subsequent reactions of the α -chromanoxyl and γ -chromanoxyl radicals. For example, the pool of the α -T radicals will couple with 4-nitrobenzyl radical whereas a substantial portion (about 20%) of γ -T radicals can dimerize and the resulting dimer further reacts with 4-nitrobenzyl chloride. Electron transfer is likely the rate determining step in the reactions of vitamin E with 4-nitrobenzyl chloride, and present results indicate that 5,7,8-trimethyl chromanols (i.e. α -T and α -T₃) are more reactive than the 7,8-dimethyl chromanols (i.e. γ -T and γ -T₃); this can be understood from the point of view that a methyl is an electron donating group.



Antioxidant activity of vitamin E

It is generally recognized that the antioxidant properties of vitamin E compounds are based on their ability to donate a phenolic hydrogen to a reactive radical or active oxygen species [89]. For instance, it has been demonstrated [574] that α -T can effectively scavenge carbon-centered cyclohexyl radical by direct hydrogen transfer which is a crucial

step in terminating radical reactions. Nagaoka *et al.* [443] suggested that the mechanism for the antioxidant action of vitamin E involves a charge transfer process *viz.* electron transfer followed by proton transfer, and proton tunnelling may also play an important role. Another pathway for the antioxidant action of vitamin E is that its phenoxyl radical scavenges the deleterious free radicals (i.e. peroxy, hydroperoxy or carbon radicals) and form non-radical products. For instance, the carbon-oxygen cross products derived from α -T and alkylperoxyl radical [687,698] or methyl linoleate-peroxyl radicals [700] have been isolated. In such reactions an α -T carbon-centred radical with unpaired electron at C-9 is able to scavenge the peroxyl radicals.

In the present study, stable ether products were also obtained indicating that the vitamin E chromanoxyl radical could capture a carbon-centred benzyl radical. Under a basic condition, the tocopherol and tocotrienol phenolic anions may act as antioxidants by electron transfer mechanism *viz.* they donate an electron to a potential radical-generating compound and subsequently will trap the resulting active radical (refer Scheme 2.6). The donation of an electron from α -T or α -T₃ phenolic anion to nitroaromatic compounds gives rise to anion radical which can encounter dissociation of a leaving group followed by radical coupling processes. Present results suggest that whether the antioxidant action of α -T will be effective or not is largely depend on the nature of the counterpart reactant (i.e. the electron acceptors); the fate of radicals generated from 4-nitrobenzyl chloride and triphenylmethyl chloride are apparently different, 4-nitrobenzyl radical will undergo mainly coupling process with α -tocopheroxyl radical whereas generation of a triphenylmethyl radical can be initiated by vitamin E but not trapped by the vitamin E radical.

In conclusion, in addition to the commonly accepted antioxidant mechanism in a biological system involves hydrogen transfer from vitamin E, present studies suggest that electron transfer reaction involving phenolic anion is possibly another mode of the vitamin E's antioxidant action in biological redox systems.